

## Zonulin Serum Level in Adolescents with Bipolar I Disorder and Schizophrenia

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### WHAT IS ALREADY KNOWN ON THIS TOPIC?

- The intestinal barrier and gut–brain axis have been increasingly linked to psychiatric disorders, including mood and psychotic illnesses.
- Zonulin is a biomarker reflecting intestinal permeability, and abnormal levels have been reported in several adult psychiatric and inflammatory conditions.
- Evidence about the role of zonulin in adolescents with bipolar disorder or schizophrenia is very limited and often inconsistent.

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### ABSTRACT

**Objective:** Evidence on the contribution of intestinal permeability to the pathophysiology of bipolar disorder (BD) and schizophrenia remains limited. Zonulin, a non-invasive biomarker of intestinal permeability, has been suggested as a factor influencing psychiatric outcomes. This study investigated whether adolescents with BD-I or schizophrenia demonstrate altered serum zonulin levels and whether these levels are associated with symptom severity.

**Methods:** A case-control study was conducted involving 45 adolescents with BD-I, 45 with schizophrenia, and 45 healthy controls. Symptom severity was evaluated using the Young Mania Rating Scale and Beck Depression Inventory in BD patients, and the Positive and Negative Syndrome Scale in schizophrenia patients. The General Health Questionnaire-28 was applied to controls to exclude psychiatric morbidity. All participants also completed the Beck Suicide Inventory, the Suicide Risk-Adolescent Version Modified, and the Snaith-Hamilton Pleasure Scale.

**Results:** Serum zonulin levels were significantly lower in BD-I patients compared with controls, whereas no significant differences were observed between the schizophrenia and control groups ( $P = .066$ ) or between the BD-I and schizophrenia groups ( $P = .297$ ). Mean zonulin levels were highest among controls, intermediate in schizophrenia, and lowest in BD-I. Across both clinical groups, higher serum zonulin concentrations correlated positively with greater symptom severity and suicidal behavior. In schizophrenia, but not in BD-I, zonulin levels were additionally associated with anhedonia.

**Conclusion:** Serum zonulin does not appear to be a diagnostic biomarker for BD-I or schizophrenia. Nevertheless, it may have clinical relevance given its associations with symptom severity, suicidal behavior, and anhedonia in schizophrenia.

**Keywords:** Adolescent, brain axis, gut, intestinal permeability, mental disorders, zonulin

### INTRODUCTION

Accumulating evidence indicates that disturbances in gut microbiota composition and function are implicated in the pathogenesis of several neuropsychiatric disorders, supporting the concept that intestinal microorganisms may influence mental health through the gut–brain axis.<sup>1-3</sup> This complex, bidirectional communication network encompasses neuronal, endocrine, and immune pathways, in addition to microbial metabolic activities such as tryptophan metabolism and short-chain fatty acid

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## WHAT THIS STUDY ADDS ON THIS TOPIC?

- *This is among the first studies to compare serum zonulin levels in adolescents with bipolar I disorder, schizophrenia, and healthy controls.*
- *It shows that bipolar I adolescents have significantly lower serum zonulin levels than healthy peers, while schizophrenia patients show intermediate values.*
- *Higher zonulin concentrations are linked to greater symptom severity and suicidal behavior, and to anhedonia in schizophrenia, suggesting zonulin may reflect illness activity rather than serve as a diagnostic marker.*

production.<sup>2</sup> Notably, the gastrointestinal system produces the majority of the body's serotonin and plays a pivotal role in modulating vagal signaling and preserving blood–brain barrier integrity.<sup>3</sup>

Beyond neuromodulatory functions, the gut microbiota exerts a major influence on immune homeostasis, and disruption of this ecosystem may heighten susceptibility to oxidative stress and inflammatory processes.<sup>4</sup> Given the established contribution of immune dysregulation to the pathophysiology of bipolar disorder (BD) and schizophrenia, growing attention has been directed toward the gut, which constitutes the largest immune organ in the human body.<sup>4</sup> A prominent hypothesis proposes that microbial dysbiosis resulting from compromised intestinal barrier integrity (“leaky gut”) facilitates translocation of microbial components, leading to systemic immune activation and, consequently, neuropsychiatric manifestations.<sup>1,2</sup> Nevertheless, the precise role of intestinal permeability in the biological mechanisms underlying BD and schizophrenia is yet to be fully elucidated.<sup>3</sup>

Adolescence (12–18 years) represents a particularly sensitive developmental period marked by ongoing maturation of neural circuits governing emotion regulation, cognition, and stress reactivity. This stage is also characterized by dynamic remodeling of the gut microbiota and heightened plasticity of microbiota–gut–brain interactions.<sup>5</sup> Importantly, BD and schizophrenia commonly have their onset during adolescence or early adulthood, and earlier onset has been associated with greater clinical severity, poorer functional outcomes, and a more persistent disease course.<sup>6</sup> Accordingly, investigating gut-related biological alterations during adolescence may yield critical insights into early disease mechanisms and identify potential biomarkers relevant to illness onset and progression. However, research examining intestinal permeability in adolescent psychiatric populations remains scarce, and available data regarding serum zonulin levels in adolescents with BD or schizophrenia are limited.

Zonulin is increasingly recognized as a non-invasive and biologically meaningful indicator of intestinal barrier function. It modulates epithelial tight junctions and directly regulates paracellular permeability.<sup>7</sup> Altered zonulin expression, influenced by environmental and microbial stimuli, has been associated with impaired mucosal defense and increased passage of luminal antigens into the circulation.<sup>7,8</sup> Compared with alternative markers of gut permeability, including the lactulose–mannitol test, lipopolysaccharide-binding protein, and intestinal fatty acid-binding protein, serum zonulin measurement offers practical advantages such as stability in peripheral blood, suitability for standardized enzyme-linked immunosorbent assay (ELISA) techniques, and a mechanistic link to tight junction regulation rather than indirect indices of epithelial damage or bacterial translocation. Therefore, zonulin represents a clinically relevant and mechanistically informative biomarker for assessing intestinal permeability in this context.<sup>9</sup>

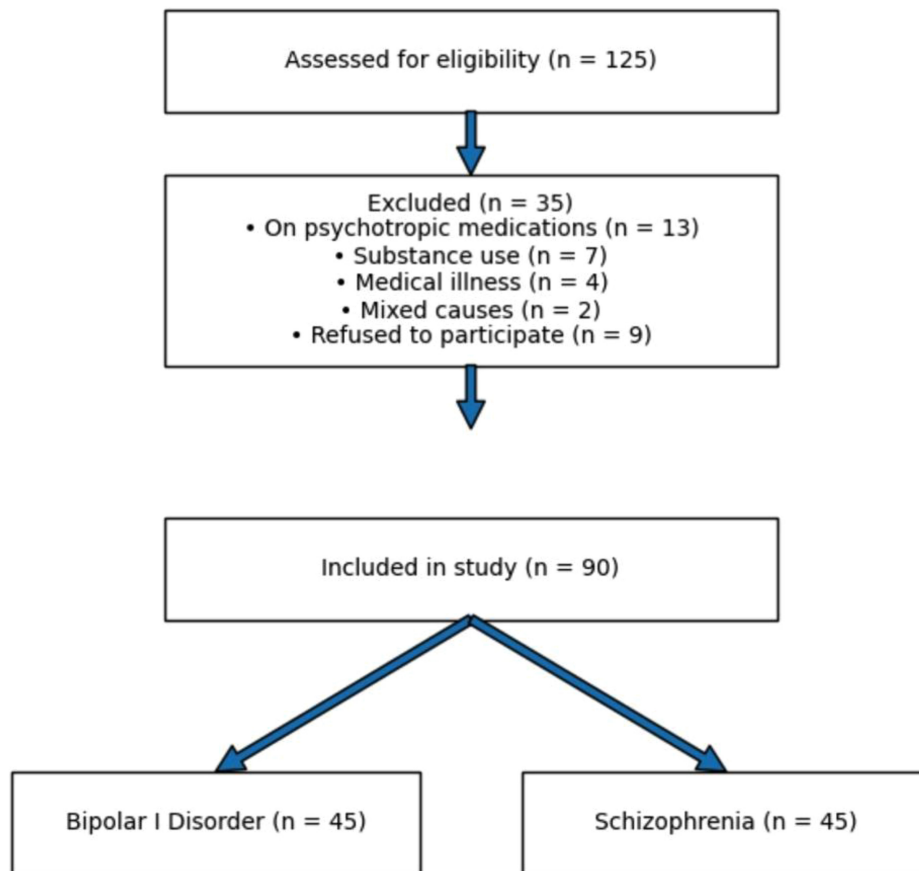
Taken together, these observations underscore a critical knowledge gap regarding intestinal barrier dysfunction in adolescents with BD-I and schizophrenia and its association with clinical symptomatology. The present study seeks to address this gap by evaluating serum zonulin levels in adolescent patients with these disorders and examining their relationships with symptom severity, thereby providing novel evidence regarding gut–brain axis involvement early in the course of illness.

It was hypothesized that serum zonulin levels would be higher in adolescents with bipolar I disorder and in adolescents with schizophrenia than in healthy controls and that increased zonulin levels would be associated with greater severity of clinical symptoms in both diagnostic groups.

## MATERIAL AND METHODS

This case–control observational study was conducted at the Psychiatry and Addiction Prevention Hospital, Kasr Al Ainy Hospitals, Faculty of Medicine, between December 2022 and September 2023. Participants were recruited from both the adolescent inpatient ward and the outpatient clinic. The study population comprised 3 groups: 45 adolescents diagnosed with bipolar I disorder (Group A), 45 adolescents diagnosed with schizophrenia (Group B), and 45 healthy volunteers serving as the control group (Group C).

A total of 125 potential participants were initially screened for eligibility. Of these, 90 participants met all inclusion criteria and were enrolled in the study. Thirty-five individuals were excluded: 13 were currently receiving psychotropic medications, 7 had current or recent substance use, 4 had significant medical illnesses, 2 were excluded due to mixed causes, and 9 declined participation. The final sample consisted of 90 participants, divided equally into 45 adolescents with bipolar I disorder and 45 with schizophrenia (Figure 1).



**Figure 1. Flowchart of recruitment of participants in the study.**

Eligible participants were adolescents aged 12-18 years of both sexes, literate, and able to complete the assessments. Diagnosis of bipolar I disorder or schizophrenia was confirmed according to DSM-5-TR criteria. Control participants were recruited through advertisements at Kasr Al Ainy Hospitals and were required to have no current or past psychiatric illness, as verified by the General Health Questionnaire-28 (GHQ-28). Written informed consent was obtained from parents or caregivers, and assent was provided by the adolescents. The study protocol received approval from the Ethics Committee of Psychiatry Department, Faculty of Medicine, Cairo University, on August 4, 2022, and was registered under MD-179-2022.

Exclusion criteria included comorbid psychiatric disorders, history of electroconvulsive therapy, or use of psychotropic medications within the preceding 3 months. Participants with neurological, metabolic, autoimmune, or neoplastic disorders, or with acute infectious diarrhea, were excluded. Those receiving immunosuppressants, probiotics, butyrate supplements, or chronic laxatives, as well as individuals who had taken antibiotics within the previous month, were also not eligible.

The 3-month medication washout period was achieved by recruiting either first-episode patients who had not yet initiated pharmacological treatment or patients who had discontinued their medications prior to presentation. This approach reflects the very poor treatment adherence commonly observed in the clinical setting of the institution. No participant was asked to discontinue effective treatment for the purpose of study participation.

The sample size was calculated based on data from prior studies,<sup>10,11</sup> yielding a minimum requirement of 135 participants with a power of

0.85 and an alpha error of 0.05. A convenience sample was obtained, consisting of 90 patients (45 with bipolar I disorder and 45 with schizophrenia) and 45 controls.

A semi-structured clinical interview derived from the Kasr Al-Ainy Psychiatry Department interview schedule was used. All interviews were conducted by the principal investigator, an assistant lecturer of psychiatry holding a master's degree in psychiatry, with formal training and clinical experience in diagnostic assessment of adolescents. The interview was used to collect sociodemographic data, clinical characteristics, and past psychiatric and medical history, and to rule out comorbid psychiatric disorders.

All participants underwent anthropometric assessment, including measurement of height and body weight using calibrated equipment, followed by calculation of body mass index (BMI) as weight in kilograms divided by height in meters squared. Laboratory investigations included overnight fasting followed by venous blood collection. A 3 mL sample was drawn from the antecubital vein, centrifuged at 3000 rpm for 10 minutes, and the serum was stored at  $-80^{\circ}\text{C}$  until analysis. Serum human zonulin concentrations were determined using a commercial ELISA kit (Human Zonulin ELISA Kit, Catalogue No. SL2712Hu, SunLong Biotech Co., Ltd.).<sup>12</sup> Concentrations were derived from a standard curve ranging from 3 to 150 ng/mL, with a sensitivity threshold of 1 ng/mL.

This ELISA kit of serum zonulin has been applied in published clinical research, including a case-control study evaluating serum zonulin and metabolic markers in school-aged children, as well as in pediatric research investigating serum biomarkers of environmental enteric

dysfunction and growth outcomes in Egyptian children.<sup>13</sup> Potential limitations of commercial zonulin ELISA kits include inter-assay variability and possible cross-reactivity with structurally related proteins, which may influence absolute concentration values. However, all samples in the present study were analyzed using the same kit lot and under identical experimental conditions, thereby minimizing systematic measurement bias and supporting valid between-group comparisons.

### Assessment Procedures

Standardized, widely used psychometric instruments with established reliability and validity were employed in this study. The Young Mania Rating Scale (YMRS) has demonstrated good reliability and validity in adolescent populations and is commonly used to assess manic symptom severity.<sup>14</sup> The Positive and Negative Syndrome Scale (PANSS) has been validated for use in adolescents with schizophrenia and related psychotic disorders.<sup>15</sup> Depressive symptoms were evaluated using the Beck Depression Inventory (BDI), which has shown strong psychometric properties in adolescent samples.<sup>16,17</sup> Suicidal ideation was assessed using the Beck Suicide Ideation Scale (BSI), which has been validated in both adolescent and adult populations.<sup>18</sup> Anhedonia was measured using the Snaith–Hamilton Pleasure Scale (SHAPS), which has demonstrated acceptable reliability in adolescents.<sup>19,20</sup> Suicide risk was further assessed using the Tool for Assessment of Suicide Risk–Adolescent Version Modified (TASR-AM), a version specifically adapted for adolescent populations.<sup>21,22</sup> Psychiatric morbidity among controls was screened using the GHQ-28, which has been validated for use in adolescents.<sup>23,24</sup> All instruments were administered using Arabic versions that have demonstrated good reliability and cultural validity in Egyptian and other Arabic-speaking populations.

### Statistical Analysis

Data were coded and analyzed using IBM SPSS Statistics version 28 (IBM Corp., Armonk, NY, USA). Categorical variables are summarized as frequencies and percentages, while continuous variables are expressed as mean, standard deviation, median, minimum, and maximum. The Kruskal–Wallis and Mann–Whitney tests were applied for group comparisons of quantitative variables,<sup>25</sup> whereas chi-square or Fisher's exact tests were used for categorical variables.<sup>26</sup> Correlations were evaluated using Spearman's correlation coefficient.<sup>27</sup> Statistical significance was set at a 2-tailed *P*-value of less than .05.

## RESULTS

### Sociodemographic and Clinical Characteristics

The mean age of participants was comparable across groups (BD: 17.07 ± 1.05 years; schizophrenia: 17.00 ± 1.10 years; controls: 17.13 ± 1.04 years; *P* = .944). Gender distribution did not differ significantly across groups (*P* = .055), nor did marital status, education, occupation, smoking, or BMI (all *P* > .18; effect sizes 0.05–0.10) (Table 1). Marital status refers to the adolescents themselves; early marriage occurs in certain social classes in Egypt, accounting for the small proportion of married participants.

### Serum Zonulin Concentrations

Serum zonulin concentrations were significantly lower in both patient groups compared with controls. Median (interquartile range (IQR)) values were BD: 8.3 (5.2–14.7) ng/mL; schizophrenia:

9.6 (6.1–16.3) ng/mL; controls: 18.2 (7.5–46.0) ng/mL, all below the reference range of 34 ± 14 ng/mL.<sup>28</sup> Differences across groups were statistically significant (Kruskal–Wallis test: *P* < .001,  $\eta^2 = 0.27$ ). Post-hoc Mann–Whitney tests revealed no significant difference between bipolar and schizophrenia groups (*P* = .297, *r* = 0.08) or between schizophrenia and controls (*P* = .066, *r* = 0.19), whereas zonulin levels were significantly lower in bipolar patients compared with controls (*P* < .001, *r* = 0.48).

### Clinical Characteristics

The mean number of suicide attempts was 1.33 (median 1, IQR 0–2) in the bipolar group and 1.60 (median 1, IQR 0–2) in the schizophrenia group (*P* = .120, *r* = 0.08). Hospitalizations averaged 1.91 (median 2, IQR 1–3) and 1.51 (median 1, IQR 1–2), respectively (*P* = .090, *r* = 0.11). Illness duration did not differ significantly (bipolar: 2.64 ± 1.96 years; schizophrenia: 2.90 ± 1.94 years; *P* = .416, *r* = 0.06). Schizophrenia symptom severity was reflected by PANSS scores (positive: 31.71 ± 9.68; negative: 28.16 ± 10.60; general psychopathology: 37.91 ± 11.32) (Table 1).

### Correlation Between Serum Zonulin and Symptom Severity

**Bipolar Disorder:** Serum zonulin levels were strongly positively correlated with YMRS scores (Spearman  $\rho = 0.62$ , 95% CI 0.43–0.76, *P* < .001) and BDI scores ( $\rho = 0.59$ , 95% CI 0.39–0.74, *P* < .001), indicating that higher zonulin concentrations were associated with more severe manic and depressive symptoms. Significant associations were also observed with suicidal behavior measures, including BSI ( $\rho = 0.60$ , 95% CI 0.40–0.75, *P* < .001) and TASR-AM ( $\rho = 0.57$ , 95% CI 0.36–0.72, *P* < .001). No significant correlation was found with anhedonia as measured by SHAPS ( $\rho = 0.19$ , 95% CI –0.06–0.41, *P* = .108). Symptom severity further correlated with suicidality, as YMRS and BDI scores each showed strong positive associations with BSI and TASR-AM (all *P* < .001). Zonulin was not significantly associated with hospitalizations (*P* = .890) or illness duration (*P* = .540) (Table 2).

**Schizophrenia:** In schizophrenia, serum zonulin levels correlated positively with suicide attempts ( $\rho = 0.61$ , 95% CI 0.41–0.76, *P* < .001) and with PANSS subscales: SAPS ( $\rho = 0.57$ , 95% CI 0.37–0.73, *P* < .001), SANS ( $\rho = 0.31$ , 95% CI 0.04–0.53, *P* = .033), and general psychopathology ( $\rho = 0.34$ , 95% CI 0.07–0.55, *P* = .013). Associations were also observed with BSI ( $\rho = 0.58$ , 95% CI 0.38–0.74, *P* < .001), TASR-AM ( $\rho = 0.55$ , 95% CI 0.34–0.71, *P* < .001), and SHAPS ( $\rho = 0.32$ , 95% CI 0.05–0.53, *P* = .013).

Subscale analyses confirmed these patterns: SAPS correlated with BSI and TASR-AM (both  $\rho > 0.56$ , *P* < .001), SANS with BSI ( $\rho = 0.26$ , *P* = .024) and TASR-AM ( $\rho = 0.25$ , *P* = .017), and general psychopathology with BSI ( $\rho = 0.33$ , *P* = .001) and TASR-AM ( $\rho = 0.30$ , *P* = .003). No correlations were observed with hospitalizations (*P* = .387) or illness duration (*P* = .811) (Table 3).

## DISCUSSION

The present study investigated serum zonulin levels in adolescents with bipolar I disorder and schizophrenia, extending prior research that has predominantly focused on adults. Adolescence (12–18 years) is a critical stage for microbiota–gut–brain interactions.<sup>5</sup> The cohort was well matched for age, gender, smoking, and BMI, reducing potential confounding influences previously noted in adult studies.<sup>10,11</sup>

Table 1. Demographic and Clinical Data in the 3 Study Groups

|  |                                  | Bipolar I Disorder Group |       | Schizophrenia Group |       | Control Group  |       | P     |
|--|----------------------------------|--------------------------|-------|---------------------|-------|----------------|-------|-------|
|  |                                  | N=45                     |       | N=45                |       | N=45           |       |       |
|  |                                  | Count                    | %     | Count               | %     | Count          | %     |       |
| Age (mean ± SD)  |                                  | 17.07 ± 1.05             |       | 17.09 ± 1.10        |       | 17.13 ± 1.04   |       | .944  |
| Sex  | Male                             | 26                       | 57.80 | 29                  | 64.40 | 18             | 40.00 | .055  |
|  | Females                          | 19                       | 42.20 | 16                  | 35.60 | 27             | 60.00 |       |
| Marital status   | Single                           | 38                       | 84.40 | 41                  | 91.10 | 41             | 91.10 | .509  |
|  | Married                          | 7                        | 15.60 | 4                   | 8.90  | 4              | 8.90  |       |
| Education  | Primary                          | 7                        | 15.60 | 6                   | 13.30 | 4              | 8.90  | .84   |
|  | High                             | 27                       | 60.00 | 30                  | 66.70 | 29             | 64.40 |       |
|  | University                       | 11                       | 24.40 | 9                   | 20.00 | 12             | 26.70 |       |
| Occupation   | Working                          | 8                        | 17.80 | 6                   | 13.30 | 5              | 11.10 | .651  |
|  | Not-Working                      | 37                       | 82.20 | 39                  | 86.70 | 40             | 88.90 |       |
| Smoking  | Smoking                          | 20                       | 44.40 | 24                  | 53.30 | 22             | 48.90 | .701  |
|  | Non smoking                      | 25                       | 55.60 | 21                  | 46.70 | 23             | 51.10 |       |
| BMI (mean ± SD)  |                                  | 27.00 ± 5.42             |       | 24.96 ± 4.33        |       | 25.51 ± 4.52   |       | .184  |
| Serum zonulin (ng/mL) (mean ± SD)  |                                  | 10.462 ± 14.239          |       | 11.104 ± 6.118      |       | 30.576 ± 44.67 |       | <.001 |
| Suicide attempts (mean ± SD)   |                                  | 1.33 ± 1.78              |       | 1.60 ± 1.36         |       |                |       | .12   |
| Number of hospital admissions (mean ± SD)                                |                                  | 1.91 ± 1.31              |       | 1.51 ± 0.92         |       |                |       | .09   |
| Duration of illness in years (mean ± SD)                                 |                                  | 2.64 ± 1.96              |       | 2.90 ± 1.94         |       |                |       | .416  |
| Young Mania Rating Scale (YMRS)  | Remission                        | 0                        | 0.00  |                     |       |                |       |       |
|  | Minimal                          | 19                       | 42.20 |                     |       |                |       |       |
|  | Mild                             | 8                        | 17.80 |                     |       |                |       |       |
|  | Moderate                         | 10                       | 22.20 |                     |       |                |       |       |
|  | Severe                           | 8                        | 17.80 |                     |       |                |       |       |
| Beck Depression Inventory (BDI)  | Normal                           | 4                        | 8.90  |                     |       |                |       |       |
|  | Mild                             | 16                       | 35.60 |                     |       |                |       |       |
|  | Moderate                         | 14                       | 31.10 |                     |       |                |       |       |
|  | Severe                           | 11                       | 24.40 |                     |       |                |       |       |
| Positive and Negative Syndrome Scale (PANSS)                             | Positive subscale                |                          |       | 31.71 ± 9.68        |       |                |       |       |
|  | Negative subscale                |                          |       | 28.16 ± 10.60       |       |                |       |       |
|  | General psychopathology subscale |                          |       | 37.91 ± 11.32       |       |                |       |       |
| Beck Suicidal Ideation Scale (BSI)                                       | Low intent                       | 19                       | 42.20 | 9                   | 20.00 |                |       | .043  |
|  | Moderate intent                  | 2                        | 4.40  | 6                   | 13.30 |                |       |       |
|  | High intent                      | 24                       | 53.30 | 30                  | 66.70 |                |       |       |
| Tool for Assessment of Suicide Risk-Adolescent Version Modified(TASR-AM) | Mild                             | 19                       | 42.20 | 10                  | 22.20 |                |       | .057  |
|  | Moderate                         | 19                       | 42.20 | 20                  | 44.40 |                |       |       |
|  | Severe                           | 7                        | 15.60 | 15                  | 33.30 |                |       |       |
| Snaith-Hamilton Pleasure Scale (SHAPS) (mean ± SD)                       |                                  | 7.56 ± 5.11              |       | 6.51 ± 5.45         |       |                |       | .386  |

No significant difference in zonulin levels between schizophrenia patients and controls was found ( $P=.066$ ), consistent with Aydın et al and Cyran et al<sup>29,30</sup> Furthermore, Misiak et al<sup>31</sup> demonstrated statistically significant decreased zonulin levels in patients with schizophrenia in comparison to controls. They explained this result by increased abundance of some bacterial strains, including *Lactobacillus* spp. and *Bifidobacterium* in patients with schizophrenia in their study. These bacterial strains have been found to decrease the levels of zonulin,<sup>32</sup> while Usta et al<sup>11</sup> and Barber et al<sup>33</sup> observed higher levels, though confounded by obesity, smoking, or lack of control groups. These inconsistencies highlight the complexity of gut-barrier dynamics in schizophrenia.

A novel finding was significantly lower zonulin levels in adolescents with BD compared with controls ( $P < .001$ ). This contrasts with adult

studies showing elevated levels.<sup>10,34</sup> Age-related differences in gut-brain axis development<sup>5</sup> and shorter illness duration in the cohort (mean 2.64 years vs. >10 years in adult studies) may explain this discrepancy. In addition, unlike prior work that included medicated patients with higher rates of obesity and smoking,<sup>10</sup> the cohort was medication-free and closely matched for BMI and smoking, minimizing these potential confounders. Notably, Aydın et al<sup>35</sup> also reported no difference in adults, underscoring variability across populations.

Importantly, zonulin levels demonstrated a significant positive correlation with symptom severity within the bipolar group, supporting its relevance as a state-related marker. The lower absolute zonulin levels observed in hospitalized BD-I adolescents likely reflect developmental and illness-stage characteristics of this early, medication-free cohort rather than an absence of association with severity. In

**Table 2. Correlations in the Bipolar I Disorder Group (45 Patients)**

|  |          | <b>Beck Suicidal Ideation Scale (BSI)</b> | <b>Tool for Assessment of Suicide Risk- Adolescent Version Modified (TASR- AM)</b> | <b>Serum Zonulin</b> |
|--|----------|---|--|----------------------|
| Number of suicide attempts   | <i>r</i> |   |  | 0.939                |
|  | <i>P</i> |   |  | <.001                |
| Number of hospital admissions  | <i>r</i> |   |  | 0.021                |
|  | <i>P</i> |   |  | .890                 |
| Duration of illness (years)  | <i>r</i> |   |  | 0.094                |
|  | <i>P</i> |   |  | .540                 |
| Young Mania Rating Scale (YMRS)  | <i>r</i> | 0.876                                     | 0.855  | 0.789                |
|  | <i>P</i> | <.001                                     | <.001  | <.001                |
| Beck Depression Inventory (BDI)  | <i>r</i> | 0.668                                     | 0.685  | 0.733                |
|  | <i>P</i> | <.001                                     | <.001  | <.001                |
| Beck Suicidal Ideation Scale (BSI)   | <i>r</i> |   | 0.920  | 0.874                |
|  | <i>P</i> |   | <.001  | <.001                |
| Tool for Assessment of Suicide Risk-Adolescent Version Modified (TASR- AM) | <i>r</i> | 0.920                                     |  | 0.918                |
|  | <i>P</i> | <.001                                     |  | <.001                |
| Snaith-Hamilton Pleasure Scale (SHAPS) (mean ± SD)                         | <i>r</i> | 0.118                                     | 0.107  | 0.243                |
|  | <i>P</i> | .441                                      | .484   | .108                 |

other words, while overall zonulin concentrations were lower compared with controls, greater symptom burden within the bipolar group was still associated with higher zonulin levels. This suggests that zonulin may function as a severity-sensitive biomarker within the adolescent bipolar spectrum, even if baseline levels differ from those reported in chronically ill adult samples.

It is noteworthy to emphasize the possible explanations accounted for the different findings in comparative analyses regarding serum zonulin level:

A. Genetic variations: zonulin is a precursor protein for haptoglobin (Hp-2), and Hp-2 levels are influenced by the Hp-2 gene.<sup>36</sup> Differences in the frequency of this gene in the studied

populations could explain the observed non-significant differences of in serum zonulin level.

- B. Measurement method: The ELISA assay used to measure zonulin might not be specific enough and could be detecting other proteins like properdin, which can lead to inaccurate results.<sup>37</sup>
- C. Gut microbiome composition: Some bacterial strains, including *Lactobacillus* spp. and *Bifidobacterium* have been found to decrease the levels of zonulin.<sup>32</sup>
- D. External factors: Several factors like diet, inflammation, stress, gliadin and medications can affect zonulin levels, potentially masking any differences between the groups.<sup>35</sup>

Researchers are struggling to understand how zonulin, a gut related marker, relates to mental health problems like schizophrenia and

**Table 3. Correlations in the Schizophrenia Group (45 Patients)**

|  |                   | <b>Beck Suicidal Ideation Scale (BSI)</b> | <b>Tool for Assessment of Suicide Risk- Adolescent Version Modified (TASR- AM)</b> | <b>Serum Zonulin</b> |
|--|-------------------|---|--|----------------------|
| Number of suicide attempts   | <i>r</i>          |   |  | 0.902                |
|  | <i>P</i>          |   |  | <.001                |
| Number of hospital admissions  | <i>r</i>          |   |  | 0.132                |
|  | <i>P</i>          |   |  | .387                 |
| Duration of illness (years)  | <i>r</i>          |   |  | 0.037                |
|  | <i>P</i>          |   |  | .811                 |
| Positive and Negative Syndrome Scale (PANSS)                               | Positive subscale | <i>r</i>                                  | 0.661  | 0.784                |
|  | Negative subscale | <i>P</i>                                  | <.001  | <.001                |
| General psychopathology subscale   | Positive subscale | <i>r</i>                                  | 0.335  | 0.355                |
|  | Negative subscale | <i>P</i>                                  | .024   | .017                 |
|  | Positive subscale | <i>r</i>                                  | 0.473  | 0.439                |
|  | Negative subscale | <i>P</i>                                  | .001   | .003                 |
| Beck Suicidal Ideation Scale (BSI)   | <i>r</i>          |   | 0.775  | 0.790                |
|  | <i>P</i>          |   | <.001  | <.001                |
| Tool for Assessment of Suicide Risk-Adolescent Version Modified (TASR- AM) | <i>r</i>          | 0.775                                     |  | 0.930                |
|  | <i>P</i>          | <.001                                     |  | <.001                |
| Snaith-Hamilton Pleasure Scale (SHAPS) (mean ± SD)                         | <i>r</i>          | 0.416                                     | 0.453  | 0.366                |
|  | <i>P</i>          | .004                                      | .002   | .013                 |

bipolar disorder. These conditions likely stem from a combination of biological, psychological, and social factors, making it difficult to pinpoint a single biomarker like zonulin as a definitive culprit. Additionally, the exact mechanisms behind these disorders remain a mystery, hindering the identification of reliable biomarkers.

While research has found biological markers potentially linked to mental illness, their usefulness in diagnosis, treatment, and especially predicting future episodes is questionable. They may not reflect brain abnormalities, and a correlation doesn't necessarily mean one causes the other. It's unclear if these abnormalities appear before symptoms or result from the illness itself. Furthermore, current diagnostic systems often categorize patients based on shared symptoms, rather than underlying causes. This can group together individuals with very different biological profiles. Treatment then focuses on managing symptoms, not necessarily addressing the root cause. This can further obscure the underlying differences between people with the same diagnosis.

In short, the complex and multifaceted nature of mental illness itself makes it difficult to find a clear link between zonulin and these disorders.

Importantly, we observed strong positive associations between serum zonulin levels and symptom severity across both disorders. In bipolar I disorder, zonulin levels correlated significantly with both YMRS and BDI scores ( $P < .001$  for both), linking intestinal permeability not only to manic activation but also to depressive symptom burden. Notably, approximately 40% of YMRS and 55% of BDI scores in our bipolar sample fell within the moderate-to-severe range, which may have enhanced our ability to detect this association. In contrast, prior studies that failed to identify such correlations often reported mean symptom scores below clinical cut-offs, potentially limiting variability and statistical sensitivity. Even among studies including moderately symptomatic patients (e.g., Aydın et al.), differences in episode type—particularly the predominance of mixed states during sampling—may partly explain discrepant findings. Our bipolar cohort consisted predominantly of individuals in manic episodes, suggesting that illness phase and symptom profile may influence the relationship between zonulin and clinical severity. These findings raise the possibility that gut-barrier dysfunction may be particularly relevant during acute mood exacerbations or in specific bipolar subgroups.

In schizophrenia, zonulin levels were positively correlated with all PANSS subscales (positive, negative, and general psychopathology), indicating a relationship with overall illness burden rather than isolated symptom domains. This broader association suggests that intestinal permeability may contribute to global neurobiological dysregulation rather than specific psychotic features alone. Previous reports have been inconsistent: some studies described negative correlations with selected PANSS items, while others found no association. Methodological differences—including smaller sample sizes, use of different symptom scales (e.g., BPRS instead of PANSS), and variability in inflammatory or cytokine profiles—may account for these discrepancies. Given that zonulin is known to increase under psychosocial stress and is partially linked to inflammatory markers such as IL-6 and IL-8, it is plausible that heightened gut permeability contributes to neuroimmune activation, which in turn exacerbates symptom severity across diagnostic categories.

Another key observation was the positive association between zonulin levels and suicidality in both patient groups. Both bipolar and

schizophrenia patients with a higher number of suicide attempts, stronger suicidal intent (BSI), and greater suicide risk (TASR-AM) demonstrated higher serum zonulin levels ( $P < .001$ ). This suggests that intestinal barrier dysfunction may be linked not only to symptom intensity but also to behavioral risk. Mechanistically, increased gut permeability may promote systemic inflammation and neuro-immune signaling alterations, processes previously implicated in suicidal behavior. While earlier studies have reported conflicting findings—ranging from elevated zonulin without risk correlation to negative or absent associations—these inconsistencies may reflect small samples of suicide attempters, heterogeneous populations, or unmeasured confounders such as smoking, alcohol use, or diet.<sup>38</sup> Importantly, our sample included a substantial proportion of individuals with past suicidal ideation or attempts, which may have provided sufficient variability to detect meaningful associations. Thus, zonulin may represent a biological correlate of suicide vulnerability in adolescents with severe psychiatric illness.

Collectively, these findings extend beyond merely demonstrating statistical associations. They suggest that gut-barrier dysfunction may be functionally linked to symptom exacerbation and suicide risk during adolescence—a developmental period characterized by ongoing maturation of the microbiota-gut-brain axis. While causality cannot be inferred, increased intestinal permeability may contribute to systemic inflammatory signaling, microglial activation, and altered neurotransmission, thereby intensifying affective and psychotic symptomatology. Future research should integrate longitudinal zonulin measurements, microbiome profiling, inflammatory biomarkers, and clinical course tracking to determine whether zonulin functions as a state marker, trait vulnerability marker, or predictor of treatment response. Such work may clarify whether targeting intestinal permeability represents a viable adjunctive strategy in adolescent psychiatry.

## CONCLUSION

Serum zonulin levels were assessed in adolescents with bipolar I disorder and schizophrenia. Bipolar patients showed significantly lower levels than controls, while schizophrenia cases did not differ. Across both disorders, higher zonulin was linked to greater symptom severity and suicidality, suggesting it may serve as a peripheral biomarker connecting intestinal permeability with psychopathology in youth. These findings underscore the importance of the gut-brain axis in adolescent mental health and highlight zonulin as a potential early indicator of illness severity. Future studies should employ longitudinal designs to track zonulin changes across mood and psychotic episodes, incorporate detailed assessments of diet, microbiota composition, and relevant genetic factors, and include larger, multi-center samples to improve generalizability. Such research could clarify causal pathways, determine whether zonulin predicts clinical outcomes, and inform targeted interventions aimed at modulating gut permeability as a therapeutic strategy.

## Limitations

Key limitations should be carefully considered when interpreting these findings. First, the cross-sectional case-control design precludes causal inference and limits conclusions regarding the temporal relationship between zonulin levels and symptom severity. It therefore remains unclear whether altered zonulin levels contribute to the pathophysiology of BD and schizophrenia or represent a consequence of acute psychopathology.

Second, the relatively small and region-specific sample size may limit generalizability and reduce statistical power, increasing the possibility of both type 1 and type 2 errors. Although statistically significant associations were identified, replication in larger, multi-center cohorts is necessary to confirm the robustness and stability of these findings.

Third, potential confounding factors cannot be fully excluded. While participants were medication-free at the time of assessment, data regarding prior psychotropic medication exposure were not systematically analyzed. Previous treatment history may influence gut permeability and serum zonulin levels, and its absence from the analysis limits interpretation of whether observed differences reflect illness-related or treatment-related effects. Additionally, unmeasured variables such as diet, gut microbiota composition, body composition beyond BMI, inflammatory status, and relevant genetic polymorphisms (e.g., haptoglobin variants) may have influenced zonulin concentrations and partially accounted for group differences.

Finally, the absence of longitudinal follow-up prevents evaluation of whether zonulin levels fluctuate across mood states, illness progression, or treatment response. Without prospective data, it is not possible to determine whether zonulin serves as a state marker, trait marker, or predictor of clinical outcomes.

Despite these limitations, the study benefits from the inclusion of a medication-free adolescent sample, the use of standardized diagnostic procedures, and careful matching of controls. Larger, longitudinal, and mechanistically oriented studies are needed to clarify causal pathways, account for treatment history, and determine the clinical utility of zonulin as a biomarker in youth psychiatric populations.

**Ethics Committee Approval:** All participants were informed of the fundamental principles governing their involvement. Participation was entirely voluntary and free of charge, with no direct benefit expected from enrollment. Participants were assured of their right to withdraw at any time without providing justification, and their identities were protected to ensure full confidentiality. While the study results may be disseminated through scientific publications, no identifying information was disclosed. The study protocol received approval from the Ethics Committee of Psychiatry Department, Faculty of Medicine, Cairo University, on August 4, 2022, and was registered under MD-179-2022.

**Artificial Intelligence Usage Statement:** The authors declared that no Artificial Intelligence tool was used in the preparation of the manuscript.

**Informed Consent:** Written informed consent was obtained from all participants, and parental assent was secured for adolescent participants. Consent included permission for the anonymized data to be used in scientific publications, with strict safeguards to preserve confidentiality.

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## REFERENCES

1. Nguyen TT, Kosciolk T, Eyer LT, et al. Overview and systematic review of studies of microbiome in schizophrenia and bipolar disorder. *J Psychiatr Res.* 2018;99:50-61. [\[CrossRef\]](#)
2. Alam R, Abdolmaleky HM, Zhou JR. Microbiome, inflammation, epigenetic alterations, and mental diseases. *Am J Med Genet B Neuropsychiatr Genet.* 2017;174(6):651-660. [\[CrossRef\]](#)
3. McGuinness A. A Systematic Review of Gut Microbiota Composition in Observational Studies of Major Depressive Disorder, Bipolar Disorder and Schizophrenia. Published Online April 12, 2022. [\[CrossRef\]](#)
4. Rudzki L, Szulc A. "Immune gate" of psychopathology-the role of gut derived immune activation in major psychiatric disorders. *Front Psychiatry.* 2018;9:205. [\[CrossRef\]](#)
5. Flannery J, Callaghan B, Sharpton T, et al. Is adolescence the missing developmental link in microbiome-gut-brain axis communication? *Dev Psychobiol.* 2019;61(5):783-795. [\[CrossRef\]](#)
6. Cirone C, Secci I, Favole I, et al. What do we know about the long-term course of early onset bipolar disorder? A review of the current evidence. *Brain Sci.* 2021;11(3):341. [\[CrossRef\]](#)
7. Demir E, Ozkan H, Seckin KD, et al. Plasma zonulin levels as a non-invasive biomarker of intestinal permeability in women with gestational diabetes mellitus. *Biomolecules.* 2019;9(1):24. [\[CrossRef\]](#)
8. Rahman MT, Ghosh C, Hossain M, et al. IFN- $\gamma$ , IL-17A, or zonulin rapidly increase the permeability of the blood-brain and small intestinal epithelial barriers: relevance for neuro-inflammatory diseases. *Biochem Biophys Res Commun.* 2018;507(1-4):274-279. [\[CrossRef\]](#)
9. Mogilevski T, Burgell R, Aziz Q, et al. Review article: the role of the autonomic nervous system in the pathogenesis and therapy of IBD. *Aliment Pharmacol Ther.* 2019;50(7):720-737. [\[CrossRef\]](#)
10. Kılıç F, Işık Ü, Demirdaş A, et al. Serum zonulin and claudin-5 levels in patients with bipolar disorder. *J Affect Disord.* 2020;266:37-42. [\[CrossRef\]](#)
11. Usta A, Kılıç F, Demirdaş A, et al. Serum zonulin and claudin-5 levels in patients with schizophrenia. *Eur Arch Psychiatry Clin Neurosci.* 2021; 271(4):767-773. [\[CrossRef\]](#)
12. SunLong Biotech Co., Ltd., Kit HZE (Catalogue No. SL2712Hu). <https://www.sunlongbiotech.com/>. Accessed July 5, 2025.
13. El Wakeel MA, El-Kassas GM, Hashem SA, et al. Serum biomarkers of environmental enteric dysfunction and growth perspective in Egyptian children. *Open Access Maced J Med Sci.* 2021;9(B):1625-1632. [\[CrossRef\]](#)
14. Young RC, Biggs JT, Ziegler VE, et al. A rating scale for mania: reliability, validity and sensitivity. *Br J Psychiatry.* 1978;133(5):429-435. [\[CrossRef\]](#)
15. Kay SR, Fiszbein A, Opler LA. The Positive and Negative Syndrome Scale (PANSS) for schizophrenia. *Schizophr Bull.* 1987;13(2):261-276. [\[CrossRef\]](#)
16. Beck AT, Ward CH, Mendelson M, et al. An inventory for measuring depression. *Arch Gen Psychiatry.* 1961;4(6):561-571. [\[CrossRef\]](#)
17. Aitchison RJ, Abu-Bader SH, Howell MK, et al. Beck Depression Inventory-II. Arabic version. In: *PsycTESTS Dataset*; 2017. [\[CrossRef\]](#)
18. Beck AT, Kovacs M, Weissman A. Assessment of suicidal intention: the scale for suicide ideation. *J Consult Clin Psychol.* 1979;47(2):343-352. [\[CrossRef\]](#)
19. Snaith RP, Hamilton M, Morley S, et al. A scale for the assessment of hedonic tone the Snaith-Hamilton Pleasure Scale. *Br J Psychiatry.* 1995;167(1):99-103. [\[CrossRef\]](#)
20. Thomas J, Al Ali M, Al Hashmi A, et al. Convergent validity and internal consistency of an Arabic Snaith Hamilton Pleasure scale. *Int Perspect Psychol.* 2012;1(1):46-51. [\[CrossRef\]](#)
21. Biddle VS. Online adolescent suicide risk assessment. *MedEdportal.* 2013. [\[CrossRef\]](#)
22. Ayoub DR, Kamel RK. *Tool for Assessment of Suicide Risk Adolescent Version Modified (TASR-AM)*. Arabic version. Mental Health Literacy; 2021. <https://mentalhealthliteracy.org/product/tool-assessment-suicide-risk-adolescent-version-modified-tasr/>.
23. Goldberg DP, Hillier VF. General health Questionnaire-28. *PsycTESTS Dataset.* 1979. [\[CrossRef\]](#)

24. Alhamad A, Al-Faris EA. The validation of the General Health Questionnaire (GHQ-28) in a primary care setting in Saudi Arabia. *J Family Community Med.* 1998;5(1):13-19. [\[CrossRef\]](#)
25. Chan YH. 102: quantitative data—parametric and non-parametric tests. *Singapore Med J.* 2003;44(8):391-396.
26. Chan YH. Biostatistics 103: qualitative data—tests of independence. *Singapore Med J.* 2003;44(10):498-503.
27. Chan YH. Biostatistics 104: correlational analysis. *Singapore Med J.* 2003;44(12):614-619. <https://cir.nii.ac.jp/crid/1370021389828557714>.
28. Heidt C, Kämmerer U, Fobker M, et al. Assessment of intestinal permeability and inflammation bio-markers in patients with rheumatoid arthritis. *Nutrients.* 2023;15(10):2386. [\[CrossRef\]](#)
29. Aydın O, Kocabaş T, Sarandöl A, et al. Comparison of plasma zonulin levels between symptom exacerbation and treatment response periods in schizophrenia: a case–control study with follow-up. *Eur Psychiatry.* 2023;66(suppl 1):S633-S633. [\[CrossRef\]](#)
30. Cyran A, Pawlak E, Piotrowski P, et al. The deficit subtype of schizophrenia is associated with a pro-inflammatory phenotype but not with altered levels of zonulin: findings from a case-control study. *Psychoneuroendocrinology.* 2023;153:106109. [\[CrossRef\]](#)
31. Misiak B, Pawlak E, Rembacz K, et al. Associations of gut microbiota alterations with clinical, metabolic, and immune-inflammatory characteristics of chronic schizophrenia. *J Psychiatr Res.* 2024;171:152-160. [\[CrossRef\]](#)
32. Veres-Székely A, Szász C, Pap D, et al. Zonulin as a potential therapeutic target in microbiota–gut–brain axis disorders: encouraging results and emerging questions. *Int J Mol Sci.* 2023;24(8):7548. [\[CrossRef\]](#)
33. Barber GS, Sturgeon C, Fasano A, et al. Elevated zonulin, a measure of tight-junction permeability, may be implicated in schizophrenia. *Schizophr Res.* 2019;211:111-112. [\[CrossRef\]](#)
34. Zengil S, Laloğlu E. Evaluation of serum zonulin and occludin levels in bipolar disorder. *Psychiatry Investig.* 2023;20(4):382-389. [\[CrossRef\]](#)
35. Aydın O, Kocabaş T, Sarandöl A, et al. Examination of plasma zonulin levels in bipolar I disorder: a case–control study with follow-up. *J Neural Transm (Vienna).* 2020;127(10):1419-1426. [\[CrossRef\]](#)
36. Maes M, Kanchanatawan B, Sirivichayakul S, et al. In schizophrenia, increased plasma IgM/IgA responses to gut commensal bacteria are associated with negative symptoms, neurocognitive impairments, and the deficit phenotype. *Neurotox Res.* 2019;35(3):684-698. [\[CrossRef\]](#)
37. Scheffler L, Crane A, Heyne H, et al. Widely used commercial ELISA does not detect precursor of haptoglobin2, but recognizes properdin as a potential second member of the zonulin family. *Front Endocrinol.* 2018;9:22. [\[CrossRef\]](#)
38. Ohlsson L, Gustafsson A, Lavant E, et al. Leaky gut biomarkers in depression and suicidal behavior. *Acta Psychiatr Scand.* 2018;139(2):185-193. [\[CrossRef\]](#)